Toxicology Excellence for Risk Assessment (TERA): Modeling the Threshold

General Comment:

It would be hard to find a chemical with more information available on which to develop an environmental risk assessment, and specifically a hazard identification and dose response assessment as describe by the U.S. Environmental Protection Agency in its formaldehyde Integrated Risk Information System (IRIS) document. Several candidates come to mind, including the recent EPA (2019)[1] evaluation of the perchlorate Reference Dose (RfD), similar to that developed by Strawson et al. (2004).[2] Here, an obvious rat thyroid carcinogen was nevertheless evaluated by all expert groups as a threshold carcinogen, and a precursor to the tumorigentic effects was modeled with a Benchmark Dose (BMD) approach and an uncertainty factor for within human variability. Another example is the chloroform assessment developed by EPA and Toxicology Excellence for Risk Assessment (TERA) in 1998 and now on IRIS.[3] Here, liver cancer was considered to develop only after a liver cell regenerative hyperplasia following cellular necrosis. Mutations were considered to also occur, generally at higher doses, but were not considered to be a key event in the tumor development. EPA scientists have also made judgments of an RfD approach for other carcinogens as described by (Hurley et al., 1998).[4] However, none of these chemicals had data in excess than what EPA has currently for formaldehyde, which a simple comparison of IRIS document sizes would readily demonstrate.

Low dose extrapolation for any carcinogen depends on a number of important parameters as per EPA (2005) cancer risk assessment guidelines. We offer comments on two of these parameters for EPA to consider, that of mode of action and choice of dosimeter. Furthermore, we strongly encourage, nay insist, that EPA follow its own guidelines on estimating risk from of a dual-MOA. Formaldehyde clearly has sufficient data to support this dual MOA. A suggested EPA approach by one of its scientists, Rory Conolly, and otherwise referred to as a hockey stick model of DPX, is the clear finesse here. It allows EPA to model cancer risk in a linear fashion at low dose, but still honors the high dose tumor response caused by a regenerative hyperplasia. It also allows EPA to model a precursor lesion, which is progressive and which also outlined in EPA (2005) guidelines.

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[1] USEPA. (2019a). Technical Support Document: Deriving a Maximum Contaminant Level Goal for Perchlorate in Drinking Water. EPA 816–R–19–007.

[2] Strawson, J., Q. Zhao and M. Dourson. 2004. Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. Reg. Tox. and Pharm. 39: 44-65

[3] See: https://iris.epa.gov/ChemicalLanding/&substance_nmbr=25.

[4] Hurley, P.M., Hill, R.N., Whiting, R.J., 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106,=437–445.

However, if EPA can somehow not accept the hockey stick model from its own, Lehman award winning scientist, then modeling the tumor response as per Dourson et al. (2008)[5] for acrylamide could be done. Simply put, consider that the controls in the formaldehyde rat tumor studies actually had a background dose of formaldehyde; estimate this background dose; model it along with other data, which will show a sharp hockey stick response; use the probit BMD model and a POD at around 1 to 2%; and then draw a straight line to a 10-6 risk. The latter choice of POD is also consistent with EPA's guideline when one has data in the low dose range (see EPA 2005, page 3-35), which one has when the "control" group is assigned a background dose. The end result of this latter approach is likely to closely approximate results from Rory's hockey stick model with DPX.

A third approach is to take the findings of a case study on an endogenous chemical, in this case formaldehyde, from the recent Alliance for Risk Assessment (*ARA*) workshop. Here, low exogenous doses of formaldehyde were shown to be not able to penetrate the cell nucleus. The findings from this case study fully support the recent publication of Thompson et al. (2020)[6] who clearly show that mutations do not occur in the tissue of interest (rat nose) and therefore a threshold approach is the appropriate judgment.

Mode of Action:

EPA and others have taken this extensive database for formaldehyde and done some remarkable assessments. For example, Health Canada took the result of a two-stage clonal growth model and developed an Exposure Point Index (EPI) and a peer-reviewed fluid-dynamic model is available developed by the Chemical Institute of Industrial Toxicology (CIIT).[7] European colleagues have reviewed this extensive database and generally developed a threshold approach to its regulation, based on a cytotoxicity with regenerative hyperplasia MOA for nasal tumor formation. EPA has also modeled these data generally using the results of one of their Lehman-award winning scientists, Rory Conolly, who compared three approaches with DNA-protein crosslinks, a J-shaped approach, an approach referred to as a hockey-stick which patterns the shaped on the tumor response in experimental animals, and an approached based on EPA's

[5] Dourson, M., Hertzberg, R., Allen, B., Haber, L., Parker, A., Kroner, O., Maier, A. and Kohrman, M. 2008. Evidence-Based Dose Response Assessment for Thyroid Tumorigenesis from Acrylamide. Regulatory Toxicology and Pharmacology 52 (2008) 264–289.

[6] Thompson CM, Gentry PR, Fitch S, Lu K, Clewell HJ III. 2020. An updated mode of action and human relevance framework evaluation for formaldehyde-related nasal tumors. Critical Reviews in Toxicology. 50:10, 919-952, DOI: 10.1080/10408444.2020.1854679.

[7] See: https://tera.org/sseus/iternew/chemdetail.php?rec=FORMALDEHYDE&hide1=0000000000000335&term1=formaldehyde&kind1=name&orgs11=0&stype1=startwith&start1=

default linear model. Starr and Swenberg (2016)[8] have also review these data and proposed a bottom-up approach to the low dose formaldehyde behavior generally based on the extensive endogenous amount of formaldehyde in everyone.

So how would one sort out this plethora of approaches? Reliance on EPA (2005) cancer guidelines is one, and highly recommended, approach. Information can also be gleaned from EPA (2007) draft guidelines on mutagenicity. What we find in both guidelines is that low dose extrapolation is not to be attempted without a review of the underlying MOA for the particular tumors of interest. For formaldehyde these tumors are potentially leukemia and definitively nasopharyngeal tumors. Not surprisingly, EPA reviewed formaldehyde's MOA information extensively.

For leukemia, EPA was not aware of information that would support any particular MOA. However and importantly, EPA also stated that an update of the epidemiology study on which their original judgment of leukemia has now indicated no statistically significant findings (page 2-44).[9] Coupled with EPA's finding that low concentrations of formaldehyde are unlikely to be distributed to distal sites (page xxi),[10] such as bone marrow, suggest to EPA, and others, that this endpoint cannot be modeled with any confidence, nor does EPA attempt to do this.

For nasal tumors EPA reviewed data on two potential MOAs, that of mutation and that of regenerative hyperplasia. Extensive evidence exists for both of these MOAs. EPA eventually judged that the MOA for mutations was (more) operative than regenerative hyperplasia and that the default linear approach to low dose extrapolation was appropriate. Furthermore, EPA considered that an adjustment to the cancer slope factor based on nasal tumors for early childhood exposure was reasonable.

However, EPA's judgment of either one MOA or another is not the only EPA approach. As described cancer risk assessment guidelines by EPA (2005, page3-22):

> Both linear and nonlinear approaches may be used when there are multiple modes of

[8] Starr, Thomas B. and James A. Swenberg. 2016. The bottom-up approach to bounding potential low-dose cancer risks from formaldehyde: An update. Regulatory Toxicology and Pharmacology 77 (2016) 167e174.

[9] "Results from the follow-up of mortality from LHP cancer in the same occupational cohort were used to derive a unit risk estimate for myeloid leukemia. In this study (see Section 2.2.2), however, there is no apparent association between myeloid leukemia mortality and cumulative exposure. A clearer association is observed with peak exposure, though it is not statistically significant in the latest follow-up (in an earlier 1994 follow-up of that study, myeloid leukemia mortality was statistically significantly associated with peak exposure; see Section 1.3.3)."

[10] "Thus, studies examining potential associations between levels of formaldehyde or formaldehyde byproducts in tissues distal to the POE (e.g., formate in blood or urine, brain formaldehyde levels) and health outcomes are not considered relevant here to interpreting the human health hazards of inhaled formaldehyde."

> action. If there are multiple tumor sites, one with a linear and another with a nonlinear mode of action, then the corresponding approach is used at each site. If there are multiple modes of action at a single tumor site, one linear and another nonlinear, then both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges. **For example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity does not occur.** [emphasis added] Modeling to a low response level can be useful for estimating the response at doses where the high-dose mode of action would be less important.

Thus, another EPA approach to the low dose extrapolation for formaldehyde, and one that we insist EPA use, is to follow its own guidelines when a chemical, such as formaldehyde, can be judged to cause tumors with two MOAs. For formaldehyde, the hockey-stick model proposed by an award winning EPA scientist, Rory Conolly, and described by EPA on pages 2-59, 2-61, 2-63, 2-68, and 2-75, would be a good candidate for this approach. This model is consistent with information on both MOAs on the formation of nasal tumors by formaldehyde as described by EPA in its formaldehyde text, in that the low dose part of the hockey stick can by hypothesized to be due to mutations, and the high dose part of the hockey stick can be hypothesized to be due to a regenerative hyperplasia. This model also results in projected lifetime cancer risks that are roughly comparable to that derived by Starr and Swenberg (2016) with their bottom up approach to mutagenic events from formaldehyde. The hockey stick model is also not inconsistent with MxGregor et al. (2006),[11] Thompson et al. (2020) or European judgments that a threshold in tumor formation by formaldehyde exists in the range of about 0.1 ppm.[12] This is supported by molecular dosimetry that unequivocally identifies 0.3 ppm as the threshold where inhaled formaldehyde no longer reaches the DNA in the nasal epithelium and, thus, cannot be mutagenic in nasal tissues. Another advantage of this approach is that EPA would be seen to model a precursor rather than an apical endpoint, which is a progressive feature of EPA's 2005 cancer guidelines.

<u>The Bottom Line on MOA</u>:

EPA should follow its own guidelines and develop a low dose cancer extrapolation based on a dual MOA. The proposed hockey-stick approach of EPA's own award winning scientist, Rory Conolly, would like approximate the end result, but other approaches might be considered, such as that suggested by Dourson et al. (2008) for acrylamide, or published by McGregor et al.

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[11] Douglas McGregor, Hermann Bolt, Vincent Cogliano, and Hans-Bernhard Richter-Reichhelm. 2006. Formaldehyde and Glutaraldehyde and Nasal Cytotoxicity: Case Study Within the Context of the 2006 IPCS Human Framework for the Analysis of a Cancer Mode of Action for Humans. Critical Reviews in Toxicology, 36:821–835. ISSN: 1040-8444 print / 1547-6898 online DOI: 10.1080/10408440600977669.

[12] See EPA Table 2-23, page 2-68 where 0.1 ppm is estimated to cause an upper bound cancer risk of 3.5×10^{-6}.

(2006) or by Thompson et al. (2020) for formaldehyde, the latter effort which was adopted by the European Union. See in particular Table 6 and Figure 8 of Thompson et al. (2020).

Choice of Dosimeter:

In its modeling of nasal tumorigenic effects for formaldehyde, EPA chose the dosimeter of cumulative dose. This choice was surprising for a number of reason, not the least of which was that the cancer risk assessment guidelines of EPA (2005) emphasize that this choice is critical in understanding the underlying MOA and necessary for any extrapolation between experimental animals and humans. That EPA's choice of cumulative dose is likely a mistake is demonstrated by EPA's description of human data in this same formaldehyde text on page 2-44, and specifically:

> Results from the follow-up of mortality from LHP cancer in the same occupational cohort were used to derive a unit risk estimate for myeloid leukemia. In this study (see Section 2.2.2), *however, there is no apparent association between myeloid leukemia mortality and cumulative exposure*. **A clearer association is observed with peak exposure, though it is not statistically significant in the latest follow-up** [emphases added] (in an earlier 1994 follow-up of that study, myeloid leukemia mortality was statistically significantly associated with peak exposure; see Section 1.3.3).

EPA also describes the develop of nasal tumors as being more likely due to peak exposures as shown on page 2-47, and specifically:

> Some of the strongest exposure-response relationships in the NCI cohort studies (Beane Freeman et al., 2013) (e.g., for NPC) **were observed for the peak exposure metric**. [emphases added] It is not clear how to extrapolate RR estimates based on peak exposure estimates to meaningful estimates of lifetime extra risk of cancer from continuous exposure to low environmental levels

Previous EPA documents also show this choice of cumulative dose to be a likely mistake. For example, EPA (1996, pages104,5) note that:

> The cumulative dose received over a lifetime, expressed as lifetime average daily dose, is generally considered an appropriate default measure of exposure to a carcinogen (Monro, 1992). The assumption is made that a high dose of a carcinogen received over a short period of time is equivalent to a corresponding low dose spread over a lifetime. While this is a reasonable default assumption based on theoretical considerations, departures from it are expected. **Another approach is needed in some cases, such as when dose-rate effects are noted (e.g., formaldehyde).** [emphasis added] Cumulative dose may be replaced, as appropriate and justified by the data, with other dose measures. In such

> cases, modifications to the default assumption are made to take account of these effects; the rationale for the elected approach is explained.

Furthermore, in a separate guideline document EPA labels formaldehyde as a category 1 gas with obvious implications for its ability to distribute throughout the body. For example, EPA (1994, page 3-38) states:

> The defining characteristic for Category 1 gases is that they are so highly water soluble and/or rapidly irreversibly reactive in the surface-liquid/tissue of the respiratory tract that they do not develop a significant backpressure (i.e., reversal in the concentration gradient at the gas-liquid interface) from the surface-liquid tissue phase during exhalation. **Category 1 gases are also distinguished by the property that the gas does not significantly accumulate in the blood which would reduce the concentration driving force and, hence, reduce the absorption rate. The default model structure is based on these characteristics. Examples of gases classified as Category 1 are hydrogen fluoride, chlorine, formaldehyde, and the volatile organic acids and esters.** [emphasis added]

Furthermore, EPA (1994, page I-6) states:

> The gases in Category 1, highly water soluble and rapidly irreversibly reactive, are distinguished by the lack of a blood-phase component to the transport resistance (i.e., almost none of the gas reaches the bloodstream), which allows the overall transport to be described by the transport resistance through air and liquid/tissue phases only (i.e., the two-phase transport resistance model). **Examples of gases in this category are hydrogen fluoride, chlorine, formaldehyde, and the volatile organic acids and esters.** [emphasis added]

The Bottom Line on Choice of Dosimeter:

EPA's choice of dosimeter is wrong. EPA's own text indicates that that the choice of dosimeter for the formation of tumors, either nasal pharyngeal or leukemia, is relate more to the peak concentration rather than the cumulative exposure. Thus, EPA needs to develop a low dose response extrapolation on the basis of peak exposure, despite the apparent difficultly in doing so. Otherwise, EPA's projected lifetime cancer risks are not credible.

EPA should also consider the recent findings of the Alliance for Risk Assessment (*ARA*) Beyond Science and Decisions workshop XIII, where formaldehyde was used as an example of a new approach to dosimetry that may offer some insights.[13] Simply put, this case study suggests that

[13] See: https://tera.org/Alliance%20for%20Risk/WorkshopXIII/Workshop_Final_Report_22.pdf, case study3, page 34.

formaldehyde cannot penetrate the cell nucleus at low concentrations, thus supporting the threshold approach suggested by Thompson et al. (2020). In such cases, EPA (2005) cancer guidelines dictate the use of RfDs or RfCs as the basis of the low dose extrapolation rather than a linear low dose modeling.